

**Kentucky Water Resources Research Institute  
Annual Technical Report  
FY 2009**

# Introduction

The 2009 Annual Technical Report for Kentucky consolidates reporting requirements of the Section 104(b) base grant award into a single document that includes: 1) a synopsis of each research project conducted with grant funds during the period, 2) citations for related publications, reports, and presentations, 3) a description of information transfer activities, 4) a summary of student support during the reporting period, and 5) notable awards and achievements during the year.

## Research Program Introduction

The activities supported by the Section 104(b) program funds and required matching are interwoven into the overall program of the Kentucky Water Resources Research Institute. Additional research, service, and technology transfer activities were funded through a variety of other sponsors. Memoranda of Agreement projects with the Kentucky Division of Water included Total Maximum Daily Load development for several Kentucky streams. Three projects were funded by the Kentucky Cabinet for Health and Family Services related to technical issues involving radiation and other contaminants at the Maxey Flats Nuclear Disposal Site and the Paducah Gaseous Diffusion Plant. The Kentucky River Authority supported watershed management services in the Kentucky River basin. The National Institute of Environmental Health Sciences supported research translation activities through the Superfund Public Outreach Program. The Kentucky Department for Environmental Protection supported 3 students through an Environmental Protection Scholarship Program coordinated by the Institute. The Division of Compliance Assistance funded support for Phase II stormwater communities.

The Kentucky Consortium for Energy and Environment, led by Lindell Ormsbee (Director of KWRI), continued a collaborative program integrating faculty and students from Murray State University and the University of Kentucky. The consortium was funded through the US Department of Energy to assist with efforts supporting a variety of environmental assessment and cleanup activities at the Paducah Gaseous Diffusion Plant.

Six student research enhancement projects were selected for support through 104(b) FY2009 funding. Projects were conducted at the University of Kentucky (3), Murray State University (1), Northern Kentucky University (1), and University of Louisville (1). Projects represented a variety of discipline areas including civil engineering (1), geology (1), biology (3), and entomology (1). The goal of this approach is to support a number of student-based efforts representing a variety of discipline areas at numerous educational institutions throughout the state to develop broad research capacity. Many state agencies are experiencing a significant loss of personnel through retirement and it is critical that undergraduate and graduate students are well trained and available to help fill this void. Project completion synopses for the six student research enhancement projects follow. An additional project to assess volunteer sampling data from the Kentucky River basin was supported with 104(b) funds and a synopsis of that project is also included in this annual report. All projects reported their results at the Kentucky Water Resources Annual Symposium on March 22, 2010.

## Elemental flow through food webs in restored and reference stream reaches

### Basic Information

<b>Title:</b>	Elemental flow through food webs in restored and reference stream reaches
<b>Project Number:</b>	2009KY124B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	KY 2nd
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Surface Water, Water Quality, Ecology
<b>Descriptors:</b>	stream restoration, secondary production, macroinvertebrates
<b>Principal Investigators:</b>	Hwa-Seong Jin

### Publication

1. Johnson, Robert C. and Hwa-seong Jin, 2010, Invertebrate Production in Restored and Reference Streams, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 63.

## Elemental Flow Through Food Webs in Restored and Reference Stream Reaches

### Problem and Research Objective

By the year 2050, current demographic models project the global population will reach 8.9 billion people (Cohen 2003). This future human population increase poses a considerable threat to freshwater resources. Consequently, a continuing need for the advancement of stream restoration science is needed to restore and conserve vital freshwater resources. Stream restoration has become an increasingly popular practice for alleviating the detrimental effects associated with anthropogenic landscape alterations. Restoration projects have increased exponentially over the past decade, and more than a billion dollars has been spent annually since 1990 (Bernhardt *et al.* 2005). Biological monitoring is an important component of stream restoration projects to ensure the initial restoration project goals have been attained. Oftentimes, however, comprehensive long-term monitoring efforts have been lacking in these projects (Bash and Ryan 2002).

Wilson Creek (restored stream) and Hart's Run (reference stream) are located in the Bernheim Research Forest (BRF) in Bullitt County, KY. Like many streams in Kentucky, Wilson Creek was historically moved into a straight channel against its valley wall, presumably to increase arable land in the stream valley. In October 2003, a 965-meter reach of Wilson Creek was relocated to its original stream channel using natural channel design techniques. Hart's Run, which flows adjacent to Wilson Creek, lies entirely within the BRF.

In this study, we used a novel post-monitoring analysis of this stream restoration project by comparing flows of carbon (C), nitrogen (N), phosphorus (P) through stream invertebrate food webs in the restored and reference streams (Fig. 1). To accomplish this, we quantified stream invertebrate community structure and secondary (hereafter 2°) production in both the reference and restored streams. We then used 2° production estimates for each taxon and gut content analysis (GCA) data to quantify food web linkages using the trophic basis of production (Benke and Wallace 1980, 1997). Finally, we used consumer-resource (hereafter C-R) stoichiometric data to determine the flows of C, N, and P through the food webs in the two streams. These analyses allowed us to not only assess the restoration project based on invertebrate community composition and food web structure (*i.e.* a structural component), but also place this information in an ecosystem-level context by addressing elemental flows through each food web.

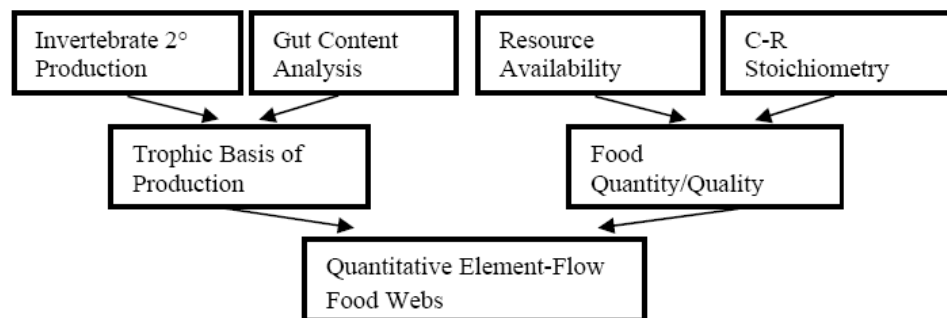


Figure 1. Flow Chart for Food Web Quantification in Restored and Reference Streams

## Methodology

**Objective 1.** Assess invertebrate 2° production and the trophic basis of production in the restored and reference streams.

**Method:** Monthly benthic macroinvertebrate samples (n=5) were collected in riffles of each reach using a Surber sampler (250 µm mesh size) for one calendar year. Samples were elutriated and rinsed through nested 2 mm and 250 µm sieves, and preserved in 10% formalin. All animals retained by the 2 mm sieve were removed and sorted by naked eye while invertebrates retained on the 250 µm sieve were subsampled following methods described by Hall *et al.* (2006) prior to removal under 15x magnification. All organisms were identified to the lowest determinable taxonomic level (genus or species for most taxa), and measured for body length to obtain ash-free dry mass using length-mass regression equations provided by Benke *et al.* (1999). Invertebrate taxa were also assigned to functional feeding groups (FFGs) following Merritt *et al.* (2007) to assess invertebrate community feeding mode changes. Kentucky macroinvertebrate biotic index (MBI, KDOW 2002) values were calculated for each sample date throughout the year. MBI scores were used to determine differences in community composition between streams.

Three different taxon-dependent methods were used to quantify macroinvertebrate production. First, production for non-tanypodinae chironomids was estimated using instantaneous growth rates provided by Walther *et al.* (2006). Production of other sufficiently abundant taxa was estimated using the size-frequency method (Hynes and Coleman 1968; Hamilton 1969; Benke and Huryn 2007) corrected for the cohort production interval (CPI) (Benke 1979, 1984). Maximum CPI for each taxon was determined from size-frequency data from this study and was verified and complemented by literature-based values. The production of rare taxa was estimated by an assumed P:B ratio, where the mean annual biomass (B) was multiplied by either 5 (univoltine species) or 10 (bivoltine species) to estimate annual production (P) (Waters 1977). Finally, crayfish production (Cambaridae) was estimated using an assumed P:B ratio of 0.58, which has been used to estimate production of these relatively long-lived taxa (Lugthart and Wallace 1992).

We completed GCA for taxa comprising greater than 1% of total 2° production at each site using digital image analysis and methods modified from Cummins (1973) to quantify the relative proportions of food resources in taxon guts. Estimates of 2° production and GCA data were then combined to determine the trophic basis of production (Benke and Wallace 1980) for each taxon.

The trophic basis of production combines measures of 2° production, GCA, and trophic efficiencies (assimilation efficiencies and production efficiencies from previous research) to estimate the total amount of each food ingested by a population per unit time (usually 1 year) along with the proportion of 2° production attributable to each food source in the diet. From this, we will create organic-matter flow food webs based on food-resource ingestion rates.

**Objective 2.** Modify food webs created in objective one to represent elemental-flow food webs from the reference and restored reaches using C-R stoichiometry.

**Method:** Invertebrate taxa and food resources (periphyton, filamentous algae, seston, leaf litter, and fine benthic organic matter (FBOM)) were sampled seasonally in each stream to quantify C-R stoichiometry. Samples of each food resource and invertebrate taxon were used to quantify the C, N, and P contents of invertebrate consumers and their food resources, along with carbon and nitrogen stable isotope ratios, in both streams. Periphyton will be sampled by scraping flat cobbles with a brush, and quantifying the area of the cobble to determine epilithic biomass per unit area. Leaf litter and filamentous algae were randomly sampled from a known area throughout the reach. Seston was sampled by filtering a known aliquot of stream water to determine the concentration of organic matter in suspension. FBOM was collected using a stovepipe corer and sampling a known area. Invertebrates were sampled qualitatively from all habitats ensuring to retrieve enough individuals for all elemental analyses. In the lab, samples were processed for analysis on the day of collection. Food resources were subsampled to determine the quantity available for consumption in each stream along with its elemental composition. Subsamples for resource quantity were dried and ashed to determine ash-free dry mass (AFDM) of each resource per unit stream area. C and N contents of food resources and invertebrate taxa were determined using a CHN analyzer and P was determined using methods modified from Rosemond (1993).

Finally, the food webs from objective one will be modified into quantitative element-flow food webs using C, N and P flows as linkage strengths between consumers and resources instead of total ingestion rates. This will be completed by multiplying the annual ingestion rate of each food resource in each consumer's diet by the food resource's %C, %N, and %P content. To determine the P flux from a resource to a consumer, for example, the ingestion rate of the resource by the consumer will be multiplied by the percent phosphorus composition of the food resource. This will give us an estimate of P ingested by the consumer from a particular food resource. By making these calculations for all linkages within each food web for C, N and P, we will be able to quantify the flows of these three elements through the entire food web.

## **Principal Findings and Significance**

Macroinvertebrate community structure (Fig. 2) and secondary production (Fig. 3) were found to be similar in the restored and reference streams approximately five years after restoration. However, there were some differences in functional feeding group production between the two streams (Fig 4.). Specifically, scraper production was higher in the restored stream and predator production was higher in the reference stream. We attribute the increased scraper production in the restored stream to increased periphyton growth resulting from increased light levels reaching the stream in the new channel where the riparian zone is relatively immature. As the riparian zone matures in the restored stream and lowers light penetration to the stream, the production of scrapers will most likely decrease. The restored stream also has a lower availability of leaf litter detritus in the stream channel throughout the year (Fig. 5). This is also most likely due to the immature riparian zone of the restored stream. As the restored stream's riparian zone

grows, we expect the amount of leaf litter available in the restored stream to become more similar to the reference stream.

We are currently analyzing the data collected for food web and nutrient flow quantification (i.e. gut contents, consumer-resource stoichiometry, etc.). Similar to our secondary production analysis, preliminary analysis suggests that there are only minor differences between these two streams in terms of food web pathways and C:N:P imbalances between macroinvertebrate consumers and their food resources.

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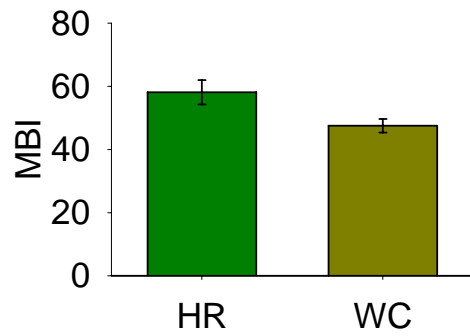


Fig. 2. Mean annual Kentucky MBI values obtained for HR (reference) and WC (restored) from monthly macroinvertebrate sampling completed in each stream.

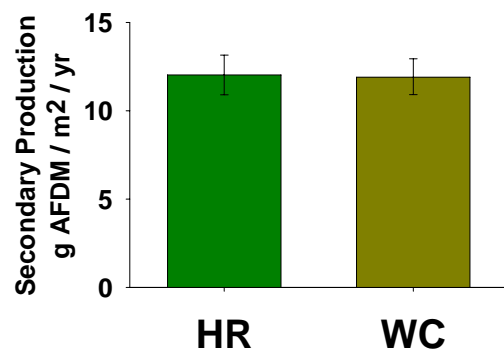


Fig. 3 Total annual secondary production for HR (reference stream) and WC (restored stream).

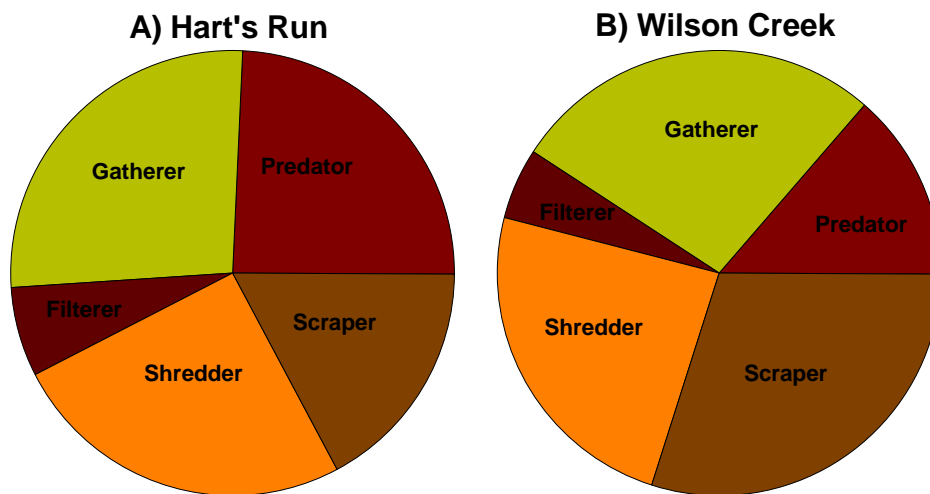


Fig. 4. Functional feeding group production in A) HR (reference) and B) WC (restored).

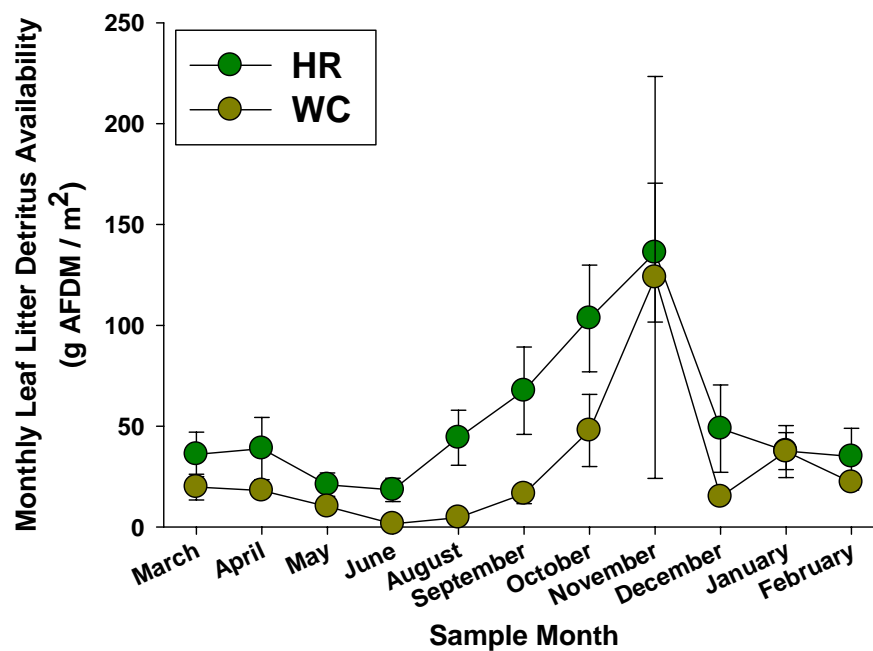


Fig. 5. Monthly leaf litter detritus availability for HR (reference) and WC (restored).

## Effects of Roundup® exposure on behavior and reproductive function in a pond-breeding salamander

### Basic Information

<b>Title:</b>	Effects of Roundup® exposure on behavior and reproductive function in a pond-breeding salamander
<b>Project Number:</b>	2009KY126B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Kentucky 1st
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Surface Water, Toxic Substances, Ecology
<b>Descriptors:</b>	amphibians, endocrine disruptors, reproductive physiology
<b>Principal Investigators:</b>	Howard H. Whiteman

### Publication

1. Aubee, C.B. and H.H. Whiteman, 2010, Effects of Roundup Exposure on Behavior and Reproductive Function in a Pond-Breeding Salamander in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 3-4.

# Effects of Roundup<sup>®</sup> Exposure on Behavior and Reproductive Function in a Pond-Breeding Salamander

## Problem and Research Objectives

Contamination of water resources by pesticides can pose serious risks to humans and wildlife. More research is needed on sublethal and low-dose effects of exposure so that action can be taken before large-scale, irreversible damage occurs. Endocrinological effects are of particular interest, as hormones drive key processes related to development, reproduction, and relative fitness. In amphibians, biocide exposure may affect reproduction and behavior by interfering with production, delivery, and/or receptor binding of hormones relevant to these processes. We utilized a pond-breeding salamander as a model to examine the acute effects of aquatic exposure to a common herbicide, Roundup<sup>®</sup>, on courtship behavior, feeding response, and plasma levels of the steroid hormones 17 $\beta$ -estradiol (E2) and testosterone (T).

## Methodology

Adult spotted salamanders (*Ambystoma maculatum*) were collected from two field sites in Calloway County and semi-randomly assigned to one of four nominal exposure concentrations: Negative Control (0.00  $\mu\text{g AI/L}$ ), Low (50.0  $\mu\text{g AI/L}$ ), Medium (500  $\mu\text{g AI/L}$ ), and High (5,000  $\mu\text{g AI/L}$ ). The Low and Medium treatments were below the maximum glyphosate concentration of approximately 2,600  $\mu\text{g AI/L}$  documented in natural habitats. All treatments were more dilute than the manufacturer's maximum recommended concentration for application. Stock solutions were prepared using Roundup<sup>®</sup> Ready-to-Use Weed and Grass Killer and stored in airtight polypropylene containers in a dark room. Because of the proprietary nature of the surfactant in Roundup<sup>®</sup>, no surfactant control was included.

Prior to exposure, specimens were maintained in well water and housed in individual containers in an environmental chamber (6°C  $\pm$ 2). At test initiation, treatment animals were individually submersed in solution for 96 hours. Control animals were newly submersed in untreated well water for an equivalent duration.

Snout-vent length, mass, and reproductive status were recorded for each specimen prior to exposure. Courtship behavior was videotaped for eight hours following treatment and was evaluated using methods from previous studies. Individual feeding response was documented as the number of mealworms eaten (maximum=10) in a 24-hour period immediately following the courtship trials.

To determine whether endocrine endpoints related to courtship and feeding response were affected by Roundup<sup>®</sup> exposure, circulating levels of 17 $\beta$ -estradiol (E2) and testosterone (T) were measured using enzyme immunoassay (EIA). Plasma was obtained from individual blood samples at the conclusion of behavioral trials and was stored at -80°C  $\pm$ 3 prior to ethyl ether extraction. Polyclonal antibodies (E2 R0008, T R156/7), enzyme-labeled hormones, and hormone standards were obtained from C. Munro (U.C. Davis).

## Principal Findings and Significance

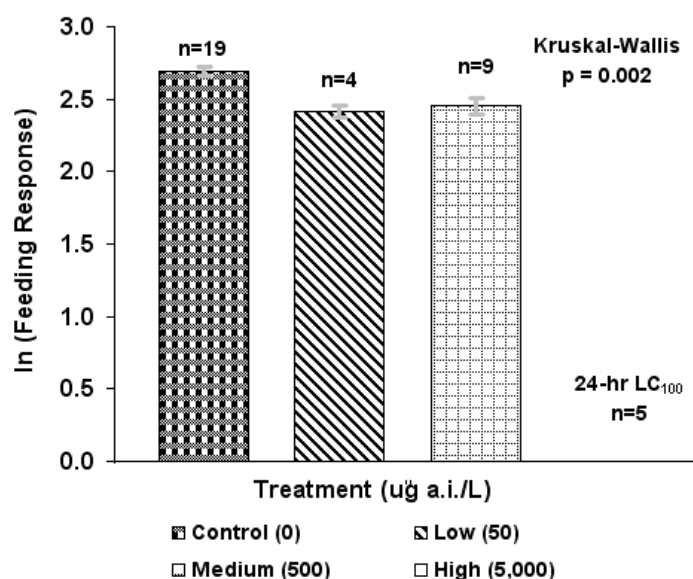
Aquatic exposure to Roundup® within the range of concentrations tested was associated with acute toxicity (*i.e.*, mortality), reduced food consumption, and increases in circulating levels of reproductive hormones. Unexpectedly, 100% mortality was noted in High treatment salamanders (only males exposed) within 24 hours of application, and 33% mortality occurred in female salamanders in the Medium treatment within 96 hours of application. An LC<sub>50</sub> could not be determined because no mortality was noted at the other treatment levels for males and females, respectively. However, the results suggest that the LC<sub>50</sub> for adult *Ambystoma maculatum* acutely exposed to Roundup® is considerably less than the LC<sub>50</sub> for amphibians exposed to the active ingredient, glyphosate, in other formulations (*eg.*, Rodeo®) and in non-formulated applications. The surfactant and/or other “inert” ingredients in Roundup® may (1) be directly toxic to the salamander and/or (2) enhance the toxicity of the active ingredient to the salamander. Alternatively, reproductive status may affect the vulnerability of adult salamanders to glyphosate exposure.

For surviving salamanders in the Low and Medium treatments, no significant differences in courtship behavior were noted. Courtship is frequently difficult to elicit in a laboratory setting, and the trials were conducted near the end of the salamander’s natural breeding season. However, preliminary analysis of log-transformed data shows that Roundup® exposure significantly decreased feeding response in male salamanders (Kruskal-Wallis  $p=0.002$ ), when controlling for mass (see Figure 1). Both treatment levels were significantly different from the Control ( $p<0.05$ ), but not from one another. Decreased foraging activity and/or effectiveness following Roundup® exposure may inhibit the salamander’s ability to re-establish energy reserves that are depleted during the breeding season.

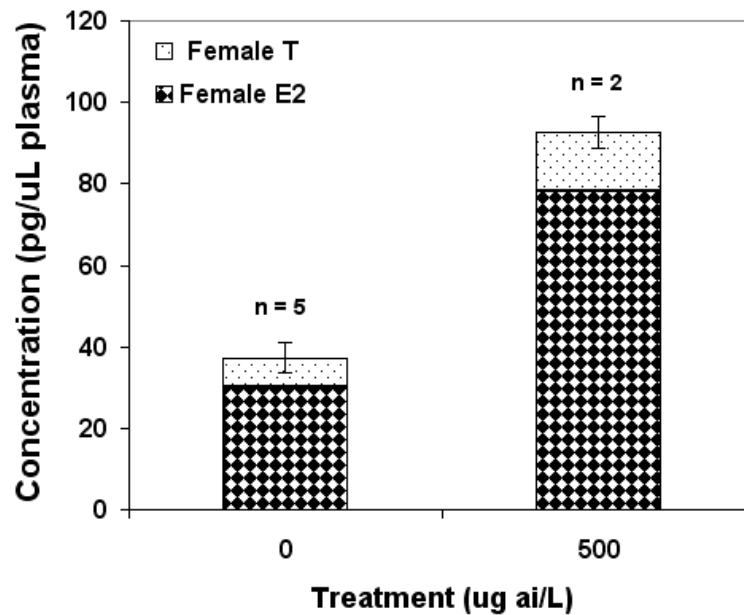
Roundup® exposure significantly increased plasma E2 levels in male salamanders, when compared to controls (mean = 31.1 pg E2/μL plasma), in the Medium treatment (mean = 168 pg E2/μL plasma,  $p < 0.05$ ) but not in the Low treatment (mean = 38.3 pg E2/μL plasma). The statistical significance of effects on females was not assessed due to low sample size in the Medium treatment ( $n=2$ ); however, the directional increase in mean plasma E2 for exposed (mean = 78.2 pg E2/μL plasma) versus unexposed (mean = 30.2 pg E2/μL plasma) females shows that an effect is plausible. E2 concentration was significantly greater in a pooled perimortem sample from three High treatment male salamanders (113 pg E2/μL plasma) than in unexposed male salamanders (mean = 31.1 pg E2/μL plasma).

Effects on plasma T were directionally similar to effects on plasma E2 but were smaller in magnitude. Roundup® exposure significantly increased plasma T in male salamanders, when compared to controls (mean = 8.65 pg T/μL plasma), at the Medium treatment level (mean = 19.6 pg T/μL plasma,  $p<0.05$ ) but not at the Low treatment level (mean = 9.28 pg T/μL plasma). A similar increase, compared to controls (mean = 6.96 pg T/μL plasma), was observed in surviving females at the Medium treatment level (mean = 14.4 pg T/μL plasma), although this is based on a single detection (28.9 pg T/μL plasma) in two samples for exposed females. Plasma T was not elevated in the pooled perimortem sample from males exposed in the High treatment (9.28 pg T/μL plasma).

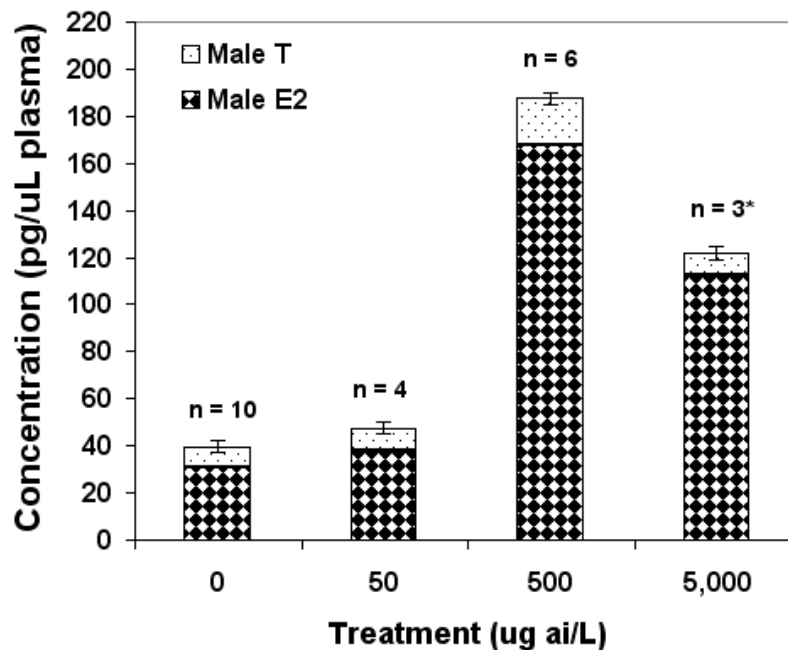
The results demonstrate that aquatic exposure to the common herbicide Roundup® may have detrimental effects on a suite of ecologically relevant endpoints in the adult spotted salamander. Although Roundup® is not labeled for direct application or discharge to water, aquatic exposure remains likely to occur, primarily as a result of runoff from target application sites. The use of other glyphosate formulations approved for aquatic use is expected to result in greater surface water concentrations of glyphosate than the terrestrial use of Roundup®. If the effects observed are related to the active ingredient and are not exclusive to the Roundup® formulation tested, the potential for direct adverse effects on amphibians and associated indirect effects on other aquatic biota may be greater than previously assessed.



**Figure 1:** Male salamanders acutely exposed to Roundup® consumed significantly fewer mealworms, compared to controls, 24 to 48 hours post-exposure (p=0.002). No significant differences were observed between the Low and Medium treatments.



**Figure 2:** Circulating levels of plasma 17β-estradiol and testosterone were increased, when compared to controls, in female salamanders acutely exposed to Roundup® at 500 µg AI/L.



**Figure 3:** Circulating levels of plasma 17β-estradiol and testosterone were significantly increased ( $p < 0.05$ ), when compared to controls, in male salamanders acutely exposed to Roundup® at 500 µg AI/L. \*Pooled sample collected perimortem from three salamanders exposed at 5,000 µg AI/L.



# Tracing the fate of Nitrogen-15 in isotopcially-labeled E. coli and determining fecal indicator die-off rates in an Inner Bluegrass karst basin, central Kentucky

## Basic Information

<b>Title:</b>	Tracing the fate of Nitrogen-15 in isotopcially-labeled E. coli and determining fecal indicator die-off rates in an Inner Bluegrass karst basin, central Kentucky
<b>Project Number:</b>	2009KY128B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Kentucky 6th
<b>Research Category:</b>	Ground-water Flow and Transport
<b>Focus Category:</b>	Groundwater, Water Quality, Methods
<b>Descriptors:</b>	karst aquifer, groundwater tracers, bacterial survival
<b>Principal Investigators:</b>	Alan Fryar

## Publications

1. Warden, John G. and Alan E. Fryar, 2010, Feasibility of using 15N-Enriched Escherichia coli as a Bacterial Tracer in the Cane Run/Royal Spring basin, Kentucky, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 73.
2. Warden, John, Alan Fryar, Gail Brion, Stephen Macko, James Ward, 2009, Bacterial Survival and Fate of 15N in Isotope-Enriched Escherichia coli in Preparation for Pathogen Tracing, in Geological Society of America Abstracts with Programs, Portland, Oregon, 41(7), p. 651.
3. Warden, John, 2010, Feasibility of using 15N-Enriched Escherichia coli as a Bacterial Tracer in the Cane Run/Royal Spring Basin, Kentucky, MS Thesis, Department of Earth and Environmental Sciences, University of Kentucky, Lexington, Kentucky, 129 p.

# **Tracing the Fate of $^{15}\text{N}$ in Isotopically-Labeled *E. coli* and Determining Fecal Indicator Die-Off Rates in an Inner Bluegrass Karst Basin, Central Kentucky**

## **Problem and Research Objectives**

Stable nitrogen isotopes have been used as a means of tracing viable *Escherichia coli* (*E. coli*) in karst environments (Ward 2008). This method, which tracks pathogen transport, is useful in determining the rates at which bacteria travel in groundwater and the remobilization capability of the bacteria within the subsurface. Application of this method within the Cane Run watershed and Royal Spring groundwater basin would provide valuable insight regarding the movement of bacteria to a spring used as a public water supply. However, before this method can be employed, careful consideration needs to be made regarding the safety of the microorganism used and its persistence in the environment. Additionally, information regarding the fate of a  $^{15}\text{N}$  spike in *E. coli* over time is essential for accurate interpretation of trace results.

## **Methodology**

A strain of wild-type *E. coli* was isolated from Royal Spring. Serological analyses and virulence testing were performed on the strain to determine its potential for pathogenicity. The wild-type *E. coli* was grown in medium enriched in  $^{15}\text{N}$  to incorporate an isotope label. The labeled *E. coli* was distributed into microcosms (including controls) containing sterilized Royal Spring water and incubated at 14° C. On testing days, a series of dilutions were prepared from the microcosms to enumerate microbial populations. Two analyses were performed on the samples. First, IDEXX Colilert® and IDEXX Quanti-Tray/2000 (IDEXX Laboratories, Westbrook, ME) were used to determine the most probable number (MPN) of *E. coli* in each microcosm. Second, IRMS was used to determine the  $^{15}\text{N}$  enrichment levels of the *E. coli*. These analyses occurred periodically for 130 days.

## **Principal Findings and Significance**

The isolated strain of *E. coli* was shown to survive for 130 days in sterilized Royal Spring water under simulated karst conditions. The concentration at day 0 was within the standard error of the concentration at day 130, and vice versa. The *E. coli* had a mean starting concentration of  $5.62 \times 10^{10}$  with a standard error of  $4.12 \times 10^9$  and a mean ending concentration of  $5.88 \times 10^{10}$  with a standard error of  $7.53 \times 10^9$ . It is expected that this strain would survive well beyond 130 days under the same conditions. Although there was statistically significant die-off from the maximum of  $1.04 \times 10^{11}$  on day 15, the day 130 concentration was different by less than one order of magnitude, indicating slow rates of die-off.

Similarly, the  $^{15}\text{N}$  label was shown to be conserved over the course of the study. Using the second sample set, there was no significant difference in  $\delta^{15}\text{N}$  values from day 1 and day 130. There was a statistical significance between days 1 and 28, but this is likely explained by a statistically significant trend in MPN data between days 8 and 15 due to the use of remnant enriched medium by the *E. coli*. No linear correlation was seen between MPN and  $\delta^{15}\text{N}$  values using the data from days 1, 28, 60, and 130. A weak positive linear correlation was seen only

using data from days 1 and 60. Figure 1 shows a comparison of *E. coli* survival and  $\delta^{15}\text{N}$  values.

The strain was serotyped O<sup>-</sup>:H<sup>-</sup> and virulence testing for factors LT, STa, STb, Stx1, Stx2, *eae*, CNF1, and CNF2 showed negative results. These results, combined with the survival and  $^{15}\text{N}$  fate results above, indicate that this strain is suitable for traces in the Cane Run/Royal Spring basin.

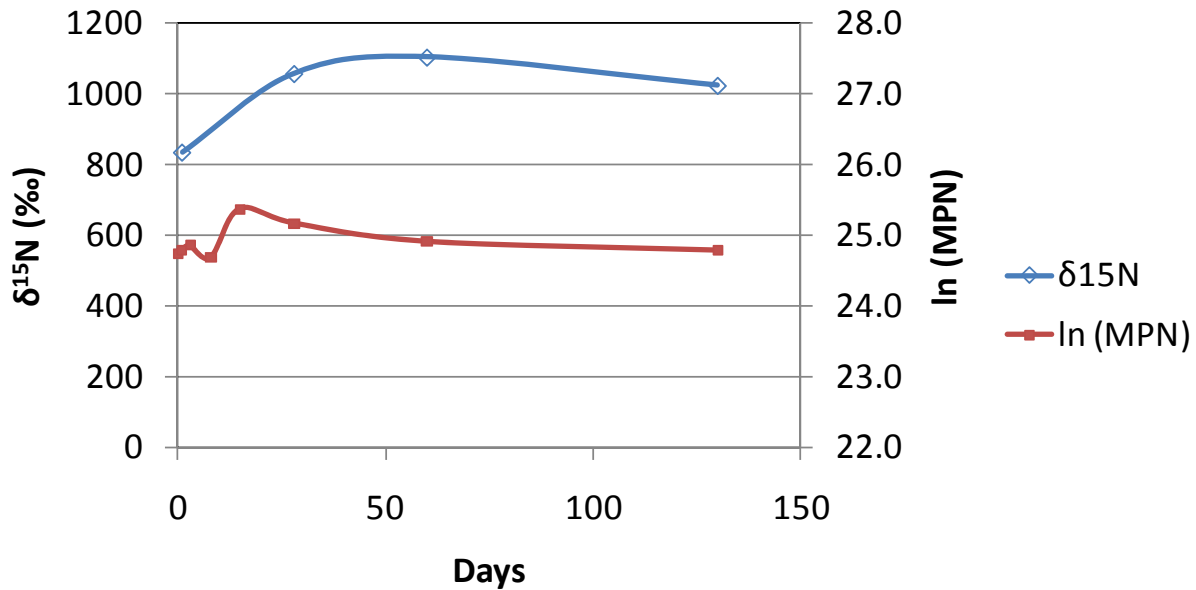


Figure 1. Comparison of *E. coli* concentration and  $\delta^{15}\text{N}$  values over the duration of the study. MPN values were transformed by natural log.

# Study of turbulent processes driving sedimentation in the rivers of Kentucky

## Basic Information

<b>Title:</b>	Study of turbulent processes driving sedimentation in the rivers of Kentucky
<b>Project Number:</b>	2009KY129B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Kentucky 6th
<b>Research Category:</b>	Engineering
<b>Focus Category:</b>	Geomorphological Processes, Sediments, Hydrology
<b>Descriptors:</b>	erosion, secondary currents, bursting
<b>Principal Investigators:</b>	James F. Fox

## Publications

1. Pulugurtha, S., J.F. Fox, and C. Harnett, 2009, The Use of the ADV and New Velocity Sensors for Studying Turbulent Processes in the Field and Laboratory, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 87-88.
2. Pulugurtha, S., J. Fox and C. Harnett, 2010, Study of Performance of Velocity Bend Sensors in Flow Over Gravel Bed Flumes and Rivers, in Proceedings of Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 81-82.

# **Study of Turbulent Processes Driving Sedimentation in the Rivers of Kentucky**

## **Problem and Research Objectives**

Sediment erosion and deposition in streams and rivers represents one of the predominant types of pollution impacting surface waters in Kentucky. Sediment pollution is harmful to aquatic communities and sediment can make filtering of water difficult for drinking purposes. Sediment can also be a problem due to filling of reservoirs or deposition in the river channel (which can decrease the volume of water available or cause flooding). This critical water problem is particularly difficult for the water resources community to solve because of the lack of accurate modeling tools for predicting the erosion of sediments. In addition, the temporary deposition of sediments and later sediment flux is difficult to predict using current modeling tools.

The research objectives were to overcome existing limitations in the literature including: (1) the linkage between secondary currents and bursting has not been studied in wide channel, rough bed conditions; (2) the existence and variability of bursting has not been quantified at high Reynolds numbers typical of streams/rivers; (3) comparison between laboratory studies at moderate Reynolds numbers with actual rivers at high Reynolds numbers has not been performed; (4) linkage between secondary currents and velocity laws have not been quantified; and (5) turbulence data to answer these shortcomings do not exist from natural streams and rivers in Kentucky.

The overall objective of this project was to study turbulent processes for a range of conditions typical of hydraulic parameters in rivers of Kentucky. To carry out this objective, measurements were performed in both the field and lab under a range of flows using traditional instruments as well as new velocity sensors that can be left *in situ*. Analysis of data were performed using traditional and advanced methods including time-average and instantaneous analyses of the flow. In order to meet the stated objectives, the following scope was outlined:

1. Develop an in-depth literature review of turbulence processes;
2. Collect lab data using two types of instruments for hydraulic conditions typical in Kentucky rivers;
3. Collect data in actual Kentucky rivers using the two types of instruments at various flow levels (ranging from low to high flows);
4. Complete time-average and instantaneous analyses of the data;
5. Perform a comparison of data from the two instruments as well as comparison of data representing laboratory and field conditions; and
6. Investigate how research results might help overcome limitations identified in the literature.

## **Methodology**

This study consisted of three primary components: (1) Data collection in the lab, (2) Data collection in the field, and (3) Analysis using decomposition techniques, auto correlation, and spectral analysis.

## Principal Findings and Significance

Past research has studied the generation, evolution, and destruction of flow structures on gravel beds along with the actual structure of turbulence. However, our understanding of turbulence structure and the processes involved are still incomplete. Most laboratory analysis of turbulent processes is carried at moderate Reynolds numbers, while in actual rivers Reynolds numbers are typically high. Kaftori et al (1994), Rodrieguz-Garcia (2008), and many other researchers have carried out experiments to study turbulence structure in the Reynold's number range 5000-11000. However, in gravel bed rivers the Reynolds number is much greater ( $> 10^4$ ). Lacey and Roy (2007) performed experiments at a Reynolds number of 200000. However, there is still need to test the validity of turbulence flow structures and laws developed in the laboratory for actual rivers.

**Table 1.** Comparison of selected studies at different Reynolds numbers.

Author of the study	Re#	Fr#	Experiment conducted in
Kaftori et al (1994)	4856 - 14010	0.71 - 1.55	Laboratory
Rodriguez and Garcia (2008)	44000 - 82000	0.45 - 0.6	Laboratory
Hurther et al (2007)	7700 - 68400	0.974 - 39.33	Laboratory
Lacey and Roy (2007)	200000	NA	River

Lab experiments were carried out in a gravel-bed flume, representative of natural rivers. Two instruments, Velocity Bend Sensors (VBS) and Acoustic Doppler Velocimeter (ADV) were used to collect instantaneous velocity data. Both instruments were set to collect velocity data at 50 Hz. The velocity bend sensors collected readings in volts while the ADV collected the velocity readings in cm/s. Velocity readings were collected by both instruments for the following range of hydraulic conditions.

**Table 2.** Hydraulic conditions for data collection in the lab.

Test #	$h$ (cm)	$Q_{\text{manning}}$ (cms)	$U_{\text{bulk}}$ (m/s)	$U^*$ (m/s)	$U_{0.5H}$ (m/s)	Fr#	Re#	$R_s$	B/H
1	7.1	0.018	0.408	0.047	0.553	0.49	27792	11.4	8.6
2	7.9	0.021	0.439	0.050	0.593	0.50	34726	12.8	7.7
3	9.0	0.026	0.473	0.052	0.638	0.51	42400	14.4	6.8
4	9.7	0.029	0.488	0.054	0.670	0.50	47563	15.7	6.3
5	10.8	0.032	0.487	0.056	0.712	0.47	52786	17.5	5.6
6	11.9	0.034	0.466	0.058	0.752	0.43	55503	19.2	5.1

Two field sites were chosen in the South Elkhorn watershed. The Ramsey site is located at Fort Spring, Kentucky. Base flow at this site is around 7 cfs. The median particle size ( $D_{50}$ ) of the pebbles found in this creek is 5.7 mm. The Gage site, located in the south Elkhorn watershed near Midway, Kentucky, has a base flow of around 50 cfs. The median particle size ( $D_{50}$ ) of pebbles found in this creek is also 5.7 mm.

Weather monitoring was done to identify possible storm events for collection of data at high flows using two velocity bend sensors calibrated in the laboratory. One velocity bend sensor was immersed vertically in the water while the other sensor was immersed at an angle. These velocity bend sensors were tied to T-posts driven into the river bed using zip-ties (the velocity bend sensors were rigidly fixed and did not move even at high flows). Data from the velocity bend sensors were collected remotely onto a laptop computer. The velocity bend sensors provide data in hexadecimal format which is stored in the laptop and later converted into velocity in m/s using conversion programs. The velocity bend sensors were programmed to collect one data point every minute. A Gurley meter was also used to collect a velocity profile of flow. The Gurley meter was fixed to a rigid rod with gradations and the point velocities at different depths were measured at a channel section downstream of the velocity bend sensors.

The mean velocity of flow was modeled by calibrating the log law using velocity data collected from the ADV. The velocities as read from the ADV probe for the different flow rates were run through a program called WIN ADV. This program filtered the raw ADV data collected and removed any bad data due to aliasing or low correlation score. The velocity data obtained after cleaning was plotted against the distance of the sampling volume (center of the probe) from the bed surface. A trend line was fitted to the resulting graph. Using the equation:

$$U_{0.5H} = u^* \left[ 8.5 + 2.5 \ln \frac{0.5H}{k_s} \right]$$

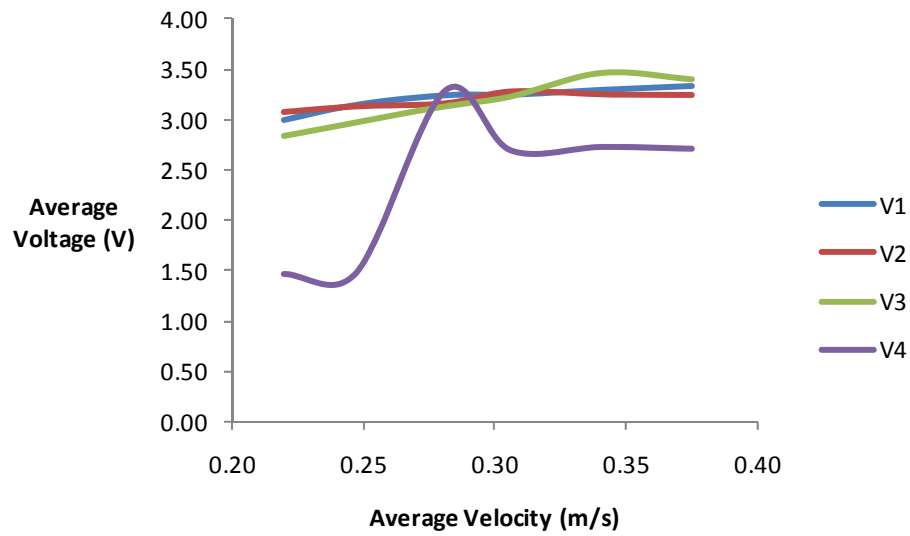
The velocities at different heights (y) in the water flow were calculated using the values,

$$u^* = (gHS)^{1/2} \text{ and } K_s = D_{84}$$

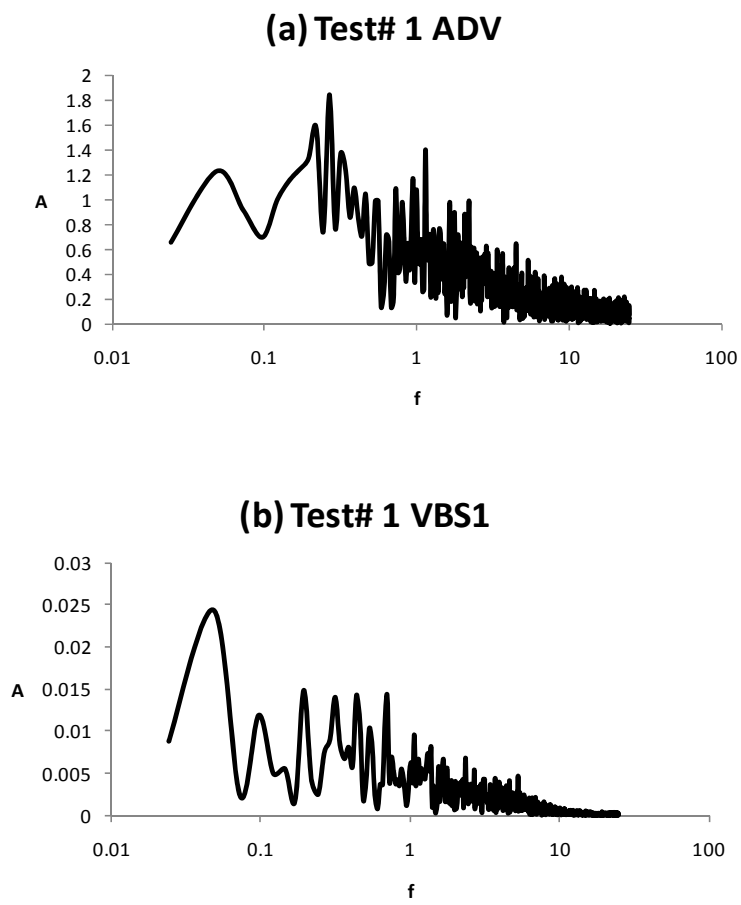
$U$  versus  $y$  calculated from the above equation were plotted on the same graph on which the ADV readings were plotted. By trial and error, values of  $K_s$  were changed until the two plots would coincide. For values of  $K_s$  where both the ADV data and the log law estimated velocities matched, we divided  $K_s$  with  $D_{50}$  and found that  $K_s = 1.8D_{50}$ . Thus the calibrated log law equation for our experimental set-up was:

$$U_{0.5H} = u^* \left[ 8.5 + 2.5 \ln \frac{0.5H}{k_s} \right]$$

The modeled velocity and average voltage were compared to find the average velocity corresponding to the average voltage read from the velocity bend sensors. An example calibration curve for the mean velocity is shown in Figure 1. V4 behaved quite differently than the other sensors for this test run due to the different length and thickness of the sensor.



**Figure 1.** Example of calibration for four different sensors.



**Figure 2.** Spectral analysis of ADV and VBS data.



Instantaneous analyses of the data were also performed. Figure 2 shows a comparison between spectral analysis performed for the ADV and VBS sensors in the laboratory. In general, the VBS captures the largest scale eddy within the flow but the sensors do not compare well for smaller scale turbulence. This was verified by comparison of turbulent intensity for the two sensors (which did not compare well) and integral length scales of the sensors (which did compare well). The primary result is that VBS sensors will work well for measuring the largest scale of turbulence in rivers, but have limitations in terms of smaller scale structure.

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Hurther, D., Lemmin, U. and Terray, E.A. (2007). "Turbulent transport in the outer region of rough-wall open-channel flows: the contribution of large coherent shear stress structures (LC3S)." *Journal of Fluid Mechanics*, 574: 465-493.

Kaftori, D., Hetsroni, G., and Banerjee, S. (1994). "Funnel-shaped vortical structure in wall turbulence." *Physics of Fluids*, 6(9), 3035-3050.

Lacey, R.W.J. and Roy, A.G. (2007). "A comparative study of the turbulent flow field with and without a pebble cluster in a gravel bed river." *Water Resources Research*, 43, W05502.

Rodriguez, J.F. and Garcia, M.H. (2008). "Laboratory measurements of 3-D flow patterns and turbulence in straight open channel with rough bed." *Journal of Hydraulic Research*, IAHR, 46(4), 454-465.

# Evaluating the impact of hemlock woolly adelgid invasion on headwater streams

## Basic Information

<b>Title:</b>	Evaluating the impact of hemlock woolly adelgid invasion on headwater streams
<b>Project Number:</b>	2009KY130B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Kentucky 5th, 6th
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Water Quality, Acid Deposition, Surface Water
<b>Descriptors:</b>	benthic macroinvertebrates, ecological function, stream chemistry
<b>Principal Investigators:</b>	Lynne Rieske-Kinney

## Publications

1. Adkins, J.K. and L.K. Rieske-Kinney, 2010, Evaluating the Impact of Hemlock Woolly Adelgid Invasion on Headwater Streams, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 1-2.
2. Adkins, J.K. and L.K. Rieske-Kinney, 2009, The Role of Eastern Hemlock on Headwater Stream Processes: Benthic and Riparian Consequences of Hemlock Woolly Adelgid Invasion, Entomological Society of America National Meeting, Indianapolis, Indiana, 13-16 December, 2009.
3. Adkins, J.K. and L.K. Rieske, 2009, Examining the Impact of Hemlock Woolly Adelgid on Headwater Streams: Preliminary Findings, 52nd Annual Southern Forest Insect Work Conference, Gulfport, Mississippi, July 2009.

# **Evaluating the Impact of Hemlock Woolly Adelgid Invasion on Headwater Streams**

## **Problem and Research Objectives**

As populations of exotic invaders establish across the landscape, important ecological functions may be compromised, potentially reducing species diversity and decreasing environmental heterogeneity. Understanding the effects these invaders have on community structure and function is critical for developing conservation plans and for restoration efforts. The objectives of our study are geared to evaluate the effects of hemlock woolly adelgid (HWA) on headwater stream quality. We are evaluating the extent to which HWA invasion affects (1) stream and riparian macroinvertebrate community dynamics, (2) stream chemistry, and (3) physical stream characteristics, including temperature, dissolved oxygen, conductivity, and turbidity. By comparing these parameters in streams with hemlock dominated riparian vegetation and streams with deciduous dominated vegetation, our study provides insight into the direct and indirect consequences of riparian eastern hemlock loss due to HWA. We also contribute to the knowledge base necessary for preservation of this threatened ecosystem.

## **Methodology**

Research sites are located at three locations in eastern Kentucky; Kentucky Ridge State Forest (Bell Co.), the Red River Gorge and Natural Bridge State Park State Nature Preserve (Meniffie, Powell, and Wolfe Counties), and the University of Kentucky's Robinson Forest (Breathitt Co.). At each site three hemlock dominated headwater streams were selected and paired with three deciduous dominated streams with similar watershed area, bankfull discharge, and conductivity. A 30 m reach was established in each study stream, and transects were established across three riffles within the reach. Benthic macroinvertebrates were sampled at 30 d intervals (March – November) from each riffle using a kick-net and Surber sampler. Artificial substrates (Hester-Dendy samplers) were utilized to monitor long-term colonization of benthic macroinvertebrates. All benthic macroinvertebrates were identified to the lowest practical taxon using appropriate keys.

Water samples were taken concurrently with macroinvertebrate sampling. Two 250 ml samples were collected ~2 m downstream from each 30 m reach, placed on ice and returned to the laboratory. Nitrate, ammonia, phosphorus, sulfate, and hardness were measured colorimetrically (Hach Corporation, Loveland, CO). Chlorophyll was measured using spectroscopic methods.

Stream velocity was measured in each stream using a flow meter, and width and depth measurements were taken to calculate total flow. Dissolved oxygen, pH, temperature, and specific conductance were measured using the YSI 556 Multi-Probe System. Daily maximum, minimum, and mean water temperature were monitored using waterproofed iButtons (Model DS1921G-F5#, Maxim Integrated Products, Sunnyvale, CA) anchored in-stream.

Alpha diversity, evenness, richness, abundance and relative abundance of benthic macroinvertebrates will be calculated to determine community composition and to evaluate changes in composition over time. Comparisons of aquatic arthropod community diversity will

be made using the Shannon-Weaver Diversity Index and Pielou's Index of Evenness while the EPT (% Ephemeroptera, Plecoptera, Trichoptera) Index and the North Carolina Biotic Index will be calculated as measures of stream quality. These indices will enable us to make comparisons in community composition between hemlock dominated and deciduous dominated headwater streams, and to evaluate changes in benthic macroinvertebrates due to adelgid-induced hemlock decline. Ultimately, a multivariate Canonical Correspondence Analysis will be used to statistically relate macroinvertebrate community parameters, riparian vegetation, and stream characteristics.

## Principal Findings and Significance

A three-way analysis of variance with location, vegetation, and season as the main effects was performed on preliminary data. The results from this initial analysis suggest that there are significant differences between hemlock and non-hemlock headwater streams in terms of water chemistry. Those differences are specifically in sulfate, calcium, total carbon, total organic carbon, and conductivity (Figure 1).

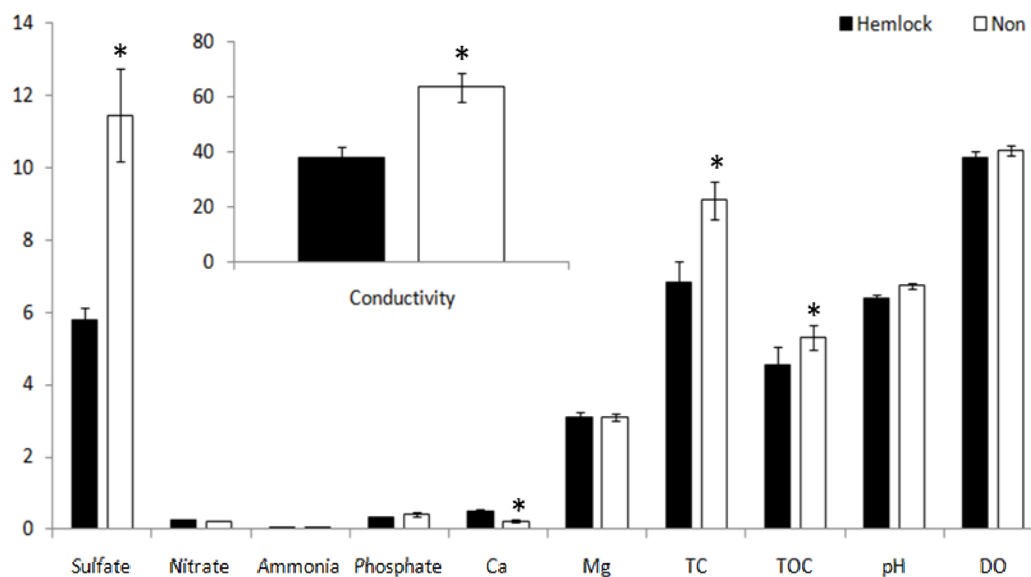


Figure 1 – Stream chemistry in hemlock and non-hemlock watersheds (\* =  $p < 0.05$ )

The preliminary analysis also suggests that abundances of benthic macroinvertebrate feeding guilds are significantly different between hemlock and non-hemlock streams. Specifically, collector-gatherers and shredders are more abundant in hemlock streams than in non-hemlock streams (Figure 2).

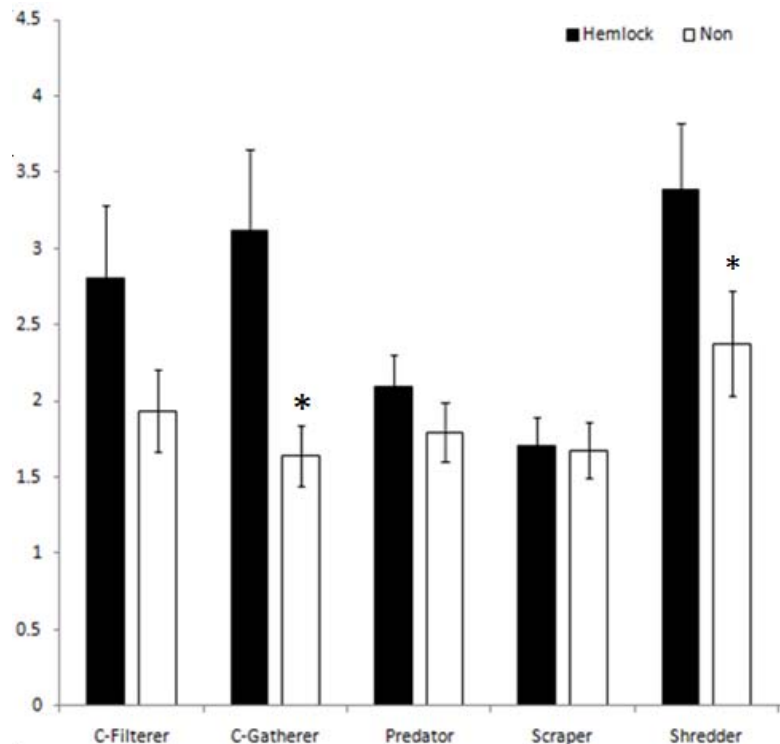


Figure 2 – Differences in benthic macroinvertebrate feeding guild abundance in hemlock and non-hemlock streams (\*  $p < 0.05$ ).

The increased abundance of shredders in hemlock streams relative to non-hemlock streams is unexpected, because most shredders utilize the autumn pulse of leaf material that enters the stream as a food source, and are typically more abundant during this time. In our “hemlock streams,” eastern hemlock is dominant or co-dominant with beech. These shredders may be opportunistically utilizing hemlock litter as a food source, leading to greater abundance. Eastern hemlock litter is small and nutrient poor, but decomposes slowly and may be available throughout the year at times when deciduous litter is unavailable or has been degraded (i.e. via nutrient leaching). Further work will be directed towards addressing questions regarding benthic shredder resource utilization and life histories.

Our results are preliminary and represent the first year of an ongoing study. This is an intensive research project that will extend beyond the KWRRRI grant funding dates. Stream monitoring began in October 2008 and is slated to continue until November 2010. The data generated from this project will represent the most intensive study on the role of eastern hemlock in headwater riparian zones in the southern Appalachians to date, and will allow us to evaluate the long-term impact of the invasion of hemlock woolly adelgid on headwater stream communities.

# Impacts of Bush honeysuckle on ephemeral aquatic ecosystems

## Basic Information

<b>Title:</b>	Impacts of Bush honeysuckle on ephemeral aquatic ecosystems
<b>Project Number:</b>	2009KY131B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Kentucky 4th
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Ecology, Acid Deposition, Surface Water
<b>Descriptors:</b>	evapotranspiration, tadpole digestion, allochthonous detritus
<b>Principal Investigators:</b>	Richard Durtsche

## Publications

1. Wallace, Andrew and Richard Durtsche, 2010, The Effects of the Invasive Amur Honeysuckle Leaf Consumption on Green Frog Tadpoles, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p 85-86.
2. Boyce, Richard and S. Lincoln Fugal, 2010, Impact of the Invasive Amur Honeysuckle (*Lonicera maackii*) on Stand Transpiration in a Wetland Forest, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p 87-88.

# Impacts of Bush Honeysuckle on Ephemeral Aquatic Ecosystems

## Problem and Research Objectives

This study addresses two aspects of the impact of the exotic invasive Bush honeysuckle (*Lonicera maackii*) on ephemeral aquatic ecosystems. Our previous investigations have indicated a high abundance of these shrubs surrounding ponds and streams in the Northern Kentucky area and decomposition rates of its leaves in water significantly faster than native tree leaves. We also found reduced digestive capacity, fitness, and survival of frog tadpoles raised in leaf teas of this invasive shrub. As teas may affect these tadpoles both internally and externally, one of the focuses of the current study is to determine whether or not *L. maackii* leaves utilized as a food source negatively impact digestion efficiency in frog tadpoles. With the encroachment of these shrubs along the waters edge of ponds and streams, our second focus is to determine if *L. maackii* has higher evapotranspiration rates than other riparian trees or shrubs, thereby providing evidence for potential reduction of water available in these ephemeral water systems.

## Methodology

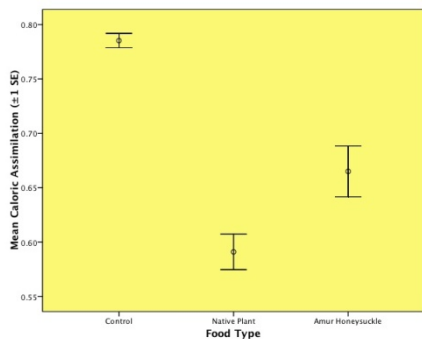
The tadpole digestion study consisted of a feeding experiment where four different concentrations of plant leaves (*L. maackii* or mixture of native riparian plants – sycamore, boxelder, silver maple) were added to an algae food base. These foods, and a no leaf control were fed to green frog tadpoles (*Lithobates clamitans*) over several weeks, and the fecal output collected. Dried food and feces were then analyzed for assimilation by the tadpoles through energy and nutrient extraction. Our initial collections of frog egg clutches and developing tadpoles in a field enclosure were vandalized by either animals or people visiting the pond. We subsequently hand collected enough tadpoles to carry out the feeding experiment in the laboratory over a four week period.

Evapotranspiration rates were monitored by measuring water movement (sap flow rates) in shrubs and trees at the St. Anne Wetlands Research and Educational Center in Melborne, KY. Sap flow was measured in trees and shrubs with thermal dissipation or Granier probes. We initially purchased eight of these probes (from Dynamax), but working with our undergraduates we developed and fabricated 12 additional Granier probes and two heat balance probes (for small shrubs) that function as well or better than the purchased probes. We also set out 20m x 30m quadrats to assess overall tree and shrub composition. These plots were established in both an old growth beech forest with few encroaching shrubs, and a secondary forest heavily invaded by *L. maackii*. Trees were cored to determine sapwood area for estimation of transpiration rates. Shrub stems were considered to be all sapwood. By doing so, we were able to assess transpiration on an areal basis for both shrubs and trees. Prior to leaf flushing at the beginning of the year we established a soil water content reflectometer at the site to measure soil moisture levels.

By using the information from the Kentucky Hydrology website, we were able to obtain stream flow rates through the wetland, and the catchment area. This information along with our evaluation of transpiration rates, vapor pressure depression, and soil water potential have been used in regression analyses to model water loss from the wetland due to evapotranspiration by *L. maackii*. As we accumulate additional data on these parameters, we will eventually be able to model the impact of *L. maackii* on this wetland system with STELLA. As yet, our database is not large enough to carry out these models, but it is the next step in our on-going study.

## Principal Findings and Significance

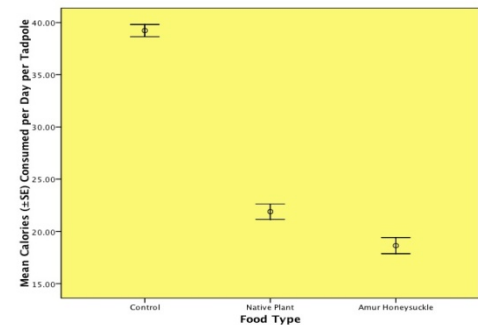
When fed diets that included leaf matter in an algae based food, tadpoles of the green frog (*Lithobates clamitans*) consumed significantly less caloric content than an algae diet alone (Fig. 1). Significantly less caloric content of *L. maackii* was consumed in comparison to native plant leaf matter. However, while caloric assimilation efficiencies were best on an algae diet (Fig. 2), more energy was extracted from *L. maackii* than native plant leaf diets, presumably offsetting the



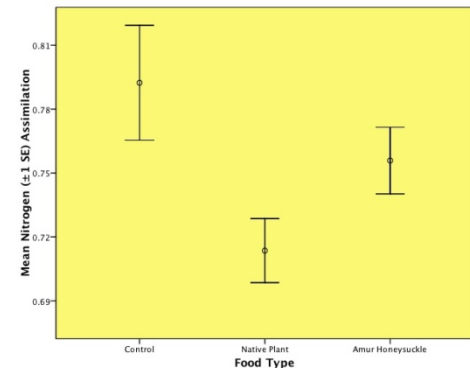
**Fig. 2.** *Lithobates clamitans* tadpole caloric assimilation over a four week period.

(Fig. 5). Evaluation of neutral detergent fiber content found that native plant leaves (74.5%) was greater than twice that of *L. maackii* (32.15%). Overall, this suggests that consumption of *L. maackii* leaf matter does not impact the digestive capacity any more than native plant leaves. However, the maximum concentrations of leaf matter in the food (10%) may not have been adequate for a definitive response.

balance of calories consumed of these two foods. Nitrogen (protein) assimilation was slightly better from *L. maackii* than native plant leaf diets, yet still best from the algae diet (Fig. 3). More potassium was extracted from either leaf diet than the algae diet (Fig. 4), and calcium assimilation was stronger from the algae and honeysuckle leaf diet than it was from the native leaf diet

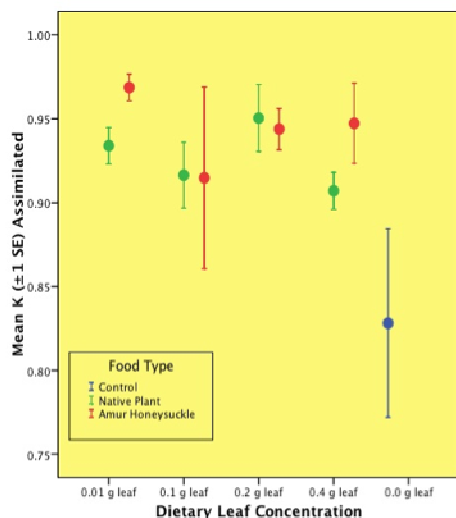


**Fig. 1.** Per day calorie consumption per tadpole of *L. clamitans* on two diets including leaf matter, and a control algae diet

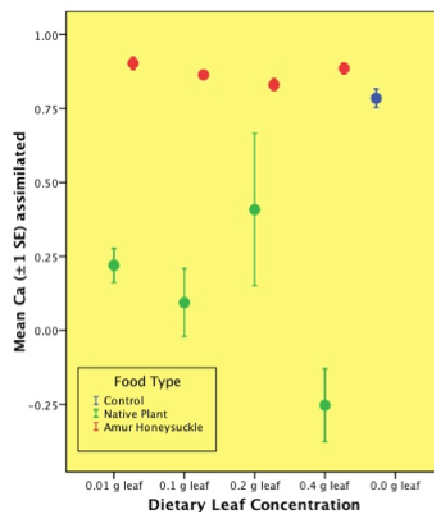


**Fig. 3.** Nitrogen (a measure of protein) assimilation was similar to the pattern found with caloric assimilation.





**Fig. 4.** The uptake of potassium was significantly greater from either dietary leaf type than the algae control food.



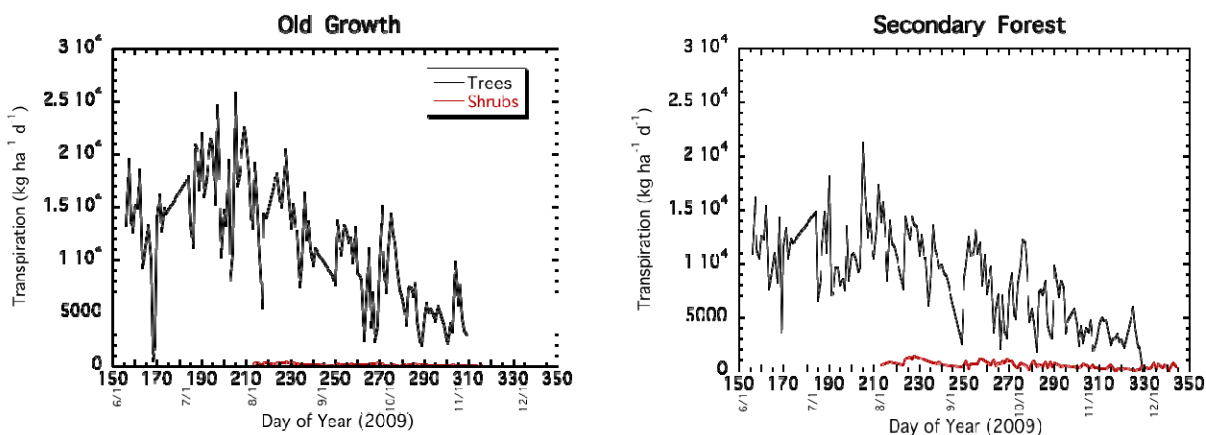
**Fig. 5.** Calcium assimilation from the control algae and from the *L. maackii* leaf foods were significantly higher than that taken up from riparian leaves.

In the evapotranspiration study, the two stands differ greatly in structure; the old growth forest has both a lower coverage of trees and of shrubs (Table 1). The *L. maackii* cover is more than 5 times greater at the secondary forest.

**Table 1.** Basal areas of trees, shrubs and *L. maackii* at the old growth and secondary forests at St. Anne Wetlands. Tree basal area also includes grape vines (*Vitis labrusca*), which account for ~1% total basal area.

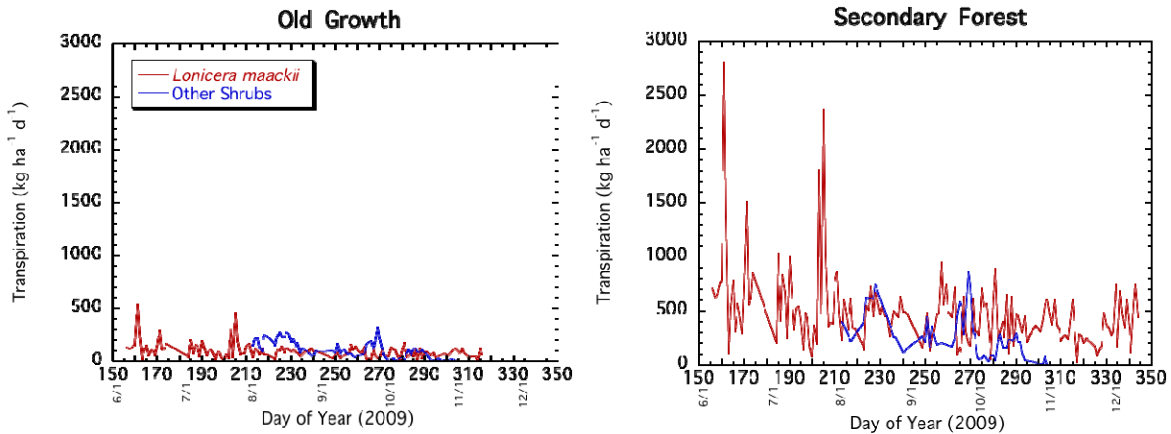
Basal Area ( $\text{m}^2 \text{ ha}^{-1}$ )	Old Growth Forest	Secondary Forest
Trees	20.3	38.9
Shrubs	0.45	2.05
<i>L. maackii</i> (% shrubs)	0.34 (75.5%)	1.76 (85.9%)

Our findings suggest that transpiration from the tree stratum is slightly higher from the old growth forest (Fig. 6), even though it has a substantially lower basal area.



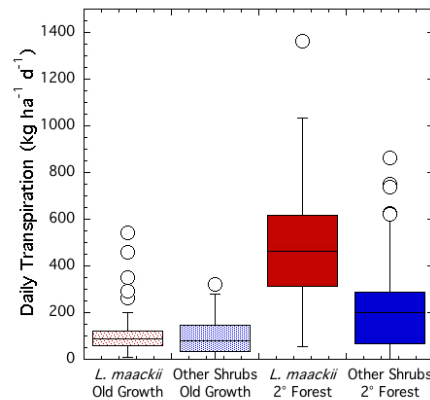
**Fig. 6.** Transpiration rates for trees and for shrubs at St. Anne Wetland. Distributions based on hourly sapflow measurements, summed for each day.

However, shrub transpiration is 3-4 times greater from the secondary forest (Fig. 7).



**Fig. 7.** Transpiration rates for shrubs at St. Anne Wetland. Distributions based on hourly sapflow measurements, summed for each day.

Shrub transpiration adds only a small amount to the total from the old growth forest (~1.4% of tree transpiration; Fig. 7). The amount added by the shrub layer at the secondary forest is much greater (~7.3% of tree transpiration). Since almost all of the shrub basal area is *L. maackii* (Table 1), over 4x the transpiration is coming from that species in comparison with the other shrubs in the secondary forests (Fig. 8).



**Fig. 8.** Boxplots of % transpiration for shrubs vs. trees at old growth and secondary forest stands at St. Anne Wetland, based on data in Fig. 1.

With a catchment area of 325,800 m<sup>2</sup> from the St. Anne Wetlands, and a stream flow rate of 26,790 m<sup>3</sup>/yr, the rainfall equivalent drainage to this area is 75.94 mm/yr. Based on the sap flow measurements, *L. maackii* has a transpiration equivalent of 7 mm/yr for this area. This suggests that Amur honeysuckle potentially reduces drainage from this wetland by approximately 9.2%. While these calculations represent average estimates across the season, the impact to ephemeral water sources in the wetland could be more extreme. This impact could drastically reduce the persistence of these ephemeral water sources and shorten the time to metamorphosis for larval amphibians.

# Ten year assessment of the Kentucky River Watershed Watch Program

## Basic Information

<b>Title:</b>	Ten year assessment of the Kentucky River Watershed Watch Program
<b>Project Number:</b>	2009KY133B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	6/30/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Kentucky 5th and 6th
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Surface Water, Hydrogeochemistry, Water Quality
<b>Descriptors:</b>	volunteer sampling, monitoring, nutrients, metals, pesticides, bacteria
<b>Principal Investigators:</b>	James A. Kipp

## Publication

1. Akasapu, Madhu and Lindell Ormsbee, 2010, Relationship Between Fecal Coliform and E. coli Values within the Kentucky River Basin, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p 89-90.

## **Ten-Year Assessment of the Kentucky River Watershed Watch Program**

### **Problem and Research Objectives**

The Kentucky River Watershed Watch (KRWV) organization was formed in 1997 to enable citizen volunteers to sample local waterways within the Kentucky River Basin and learn how to improve and protect water quality. KRWV has grown to include approximately 250 volunteers living throughout the basin, which extends over much of the central and eastern portions of the state and is home to over 710,000 Kentuckians. The watershed includes all or parts of 42 counties and drains over 7,000 square miles, with a tributary network of 15,000 miles.

Since the inception of KRWV, the Kentucky Water Resources Research Institute has assisted its volunteers with data analysis by producing a yearly summary report of water quality sampling results. A five-year analysis was completed in 2004. With the accumulation of over 10 years of sampling data, a longer term analysis and summary of KRWV sampling results through 2009 is now possible to assist the organization and its volunteers by providing further interpretation. Additionally, a survey of KRWV leadership and volunteers and resulting recommendations will help strengthen the organization and help participants better achieve their overall mission and goals.

This project compiled and statistically analyzed KRWV's water quality data collected between 1999 and 2009. The resulting summary report, titled *Kentucky River Watershed Watch: A Summary of Volunteer Water Quality Sampling Efforts in the Kentucky River Basin from 1999 to 2009*, will be distributed to KRWV volunteers via face-to-face meetings and through electronic methods (e-mail and online posting). The report includes 1) a short history of the KRWV organization; 2) an overview of sampling efforts; 3) an analysis of the annual spring, summer and fall sampling events; 4) field chemistry results; 5) a list of sampling sites and watersheds of concern; 6) how volunteers can apply their data; and 7) an assessment of the current status of the KRWV organization. The report also includes appendices describing individual water quality parameters and instructions for using the interactive, online KRWV database.

### **Methodology**

A Microsoft Access database and Microsoft Excel were used to compute statistical analyses of KRWV water quality data. For most parameters, geometric means were calculated to determine averages for the assessment period when at least three separate results were available. In order to interpret these results, graphs were created that compared the geometric means to specific water quality standards established by the state of Kentucky to protect waterways for aquatic life and human uses.

The summer pathogen samples were analyzed for fecal coliform from 1999-2007 and for *E. coli* from 2008 to 2009. In order to compare the sampling results over the entire period, a statistical analysis was conducted to determine a relationship between the two pathogen indicators. Once this relationship was established between fecal coliform and *E. coli*, a t-test was used to determine if a sampling site showed improvement (decreased pathogen levels) by comparing the 1999-2003 five-year time period with the 2004-2009 six-year time period. To perform this test, available data were converted to natural logarithms and the respective t-test

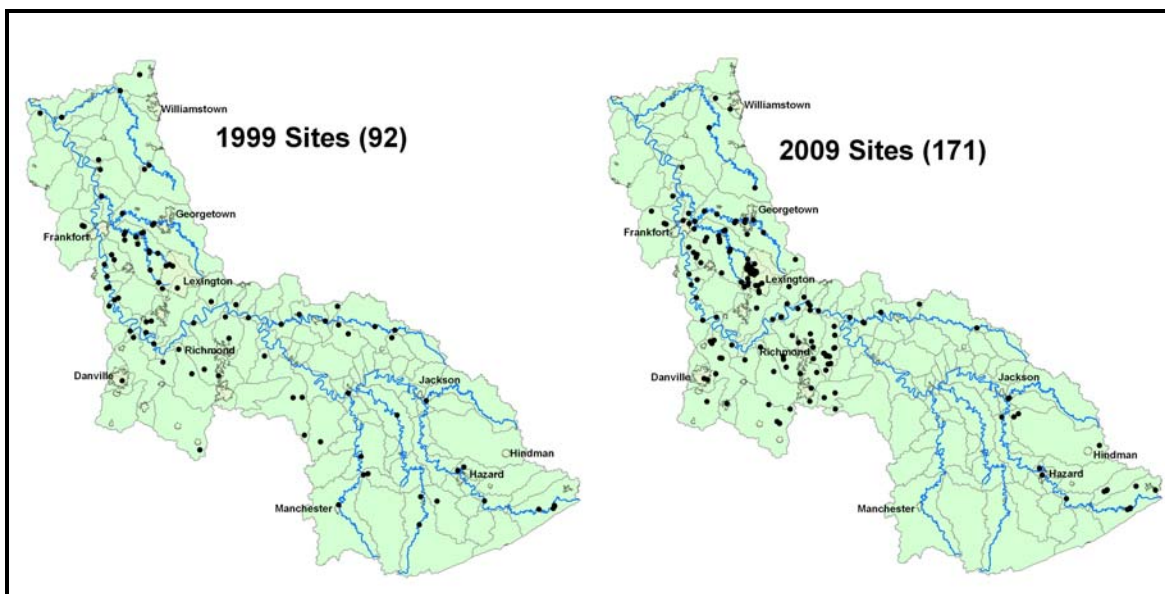
statistic was examined to evaluate if changes were statistically significant. Unfortunately, several sampling sites lacked sufficient data for such a comparative analysis.

A KRWV volunteer survey was conducted in 2009 using an online service. This survey assessed opinions on the importance of specific KRWV functions (general coordination, monitoring, training, volunteer events, subwatershed projects, and advocacy) and how well they were being performed. The survey results were compiled in an Excel spreadsheet and summarized in narrative format.

Overall data interpretations were summarized in a narrative final report, along with appropriate tables, graphs and maps. Arcview GIS applications were used to produce the maps for the report. The report, as well as the full KRWV database, will be posted on the KRWV website at [www.krww.org](http://www.krww.org) for public access.

### Principal Findings and Significance

In 1999, Kentucky River Watershed Watch volunteers sampled at 87 sites throughout the basin. After reaching a high of 248 sites in 2006, the number of sites decreased to 171 in 2009. Sampling has become increasingly more concentrated in the central region of the Kentucky River Basin, which includes the more densely populated, urbanized areas. Thus, there are currently more KRWV data results for this central region than for the upper basin areas in southeastern Kentucky or the lower basin in northern Kentucky. Figure 1 illustrates the changes in sampling site distribution from 1999 to 2009. In 2009, there were no sites located in the South and Middle Forks of the Kentucky River and only a few in the northern portion of the Kentucky River Basin.

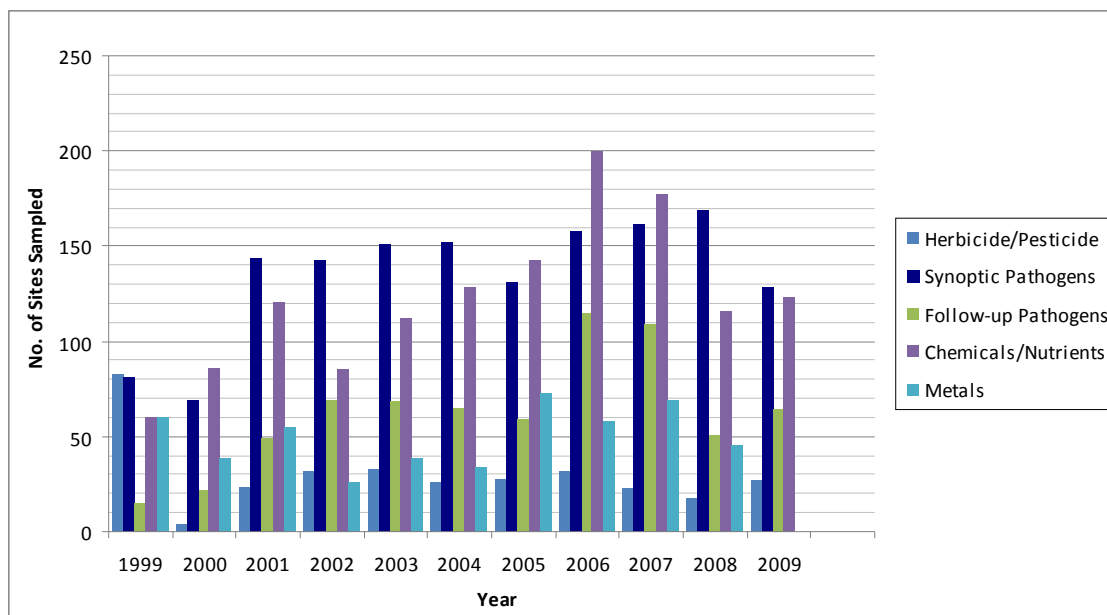


**Figure 1. Comparison of 1999 and 2009 KRWV Sampling Locations**

KRWV volunteers typically sampled the water quality at their chosen sites three to four times each year. The sampling events included herbicides and an insecticide (spring), synoptic and follow-up pathogens (twice in summer), and metals, chemistry, and nutrients (fall). Pesticides were analyzed during the spring sampling event due to the increased likelihood of

recent crop applications. Pathogens were assessed during the summer months, when people were more likely to be coming in direct contact with waterways through various recreational activities. The nutrient, chemical and metal parameters were analyzed during the fall water sampling event because of the typically lower flows observed during this time of year and the associated potential for increased concentration. In addition, samplers analyzed dissolved oxygen, pH, temperature, conductivity, and habitat condition in the field during sample collection.

Figure 2 illustrates the number of samples collected during each major sampling event from 1999 through 2009. The number of herbicide and insecticide samples that were collected are relatively low, because they were generally only assessed at newly sampled sites. Follow-up pathogen sites included only those sites where pathogen concentrations exceeded the safe swimming standard during the initial synoptic sampling event. Metals were only assessed for specific sites during the fall, low-flow sampling events.



**Figure 2. Number of Sites Sampled During Regular Sampling Events (1999-2009)**

*NOTE: No metals were assessed during 2009*

Water quality results collected during each of the annual sampling events were entered into a Microsoft Access database and assessed. This assessment was based upon averaging sampling results for sites with data from at least three of the eleven sampling years for comparison with available water quality standards. The following water quality parameters were assessed: herbicides (Metolachlor, Atrazine, 2,4-D), insecticide (Chlorpyrifos), pathogens (fecal coliform and E coli), nutrients (nitrate nitrogen, total phosphorus, sulfate), metals (antimony, arsenic, barium, beryllium, cadmium, chromium, copper, iron, lead, nickel, selenium, silver, thallium, zinc), dissolved oxygen, pH, and temperature.

This analysis of eleven years of KRWV sampling data from 1999 through 2009 provided evidence of water quality concerns throughout the Kentucky River Basin. A list of sampling sites and associated watersheds of concern was produced, along with a map showing their locations within the Basin.

In the future, this type of analysis could be strengthened by the availability of more continuous data for the individual sampling sites. By sampling regularly year-to-year, volunteers will be more likely to gain valuable insights to the current status and changes of water quality at their chosen sites. For many of the sampling parameters, water quality issues were most evident in the central region of the Kentucky River Basin. Although these findings are instructive, it should be noted that a disproportionate share of the KRWV sampling sites are located in this region.

This analysis of KRWV water quality results is intended to provide KRWV volunteers and organizers with information to guide continued sampling, focused sampling, and water quality improvement efforts. Additional sampling data from future sampling years will be useful to build on this analysis and strengthen its conclusions.

## **Information Transfer Program Introduction**

Information transfer activities are an important part of the overall program of the Kentucky Water Resources Research Institute. There are two main components, an annual symposium and the institute web site (including an electronic newsletter). The institute also participates in and supports numerous other minor technology and information transfer activities throughout the year.



## Kentucky Information Transfer Activities

### Basic Information

<b>Title:</b>	Kentucky Information Transfer Activities
<b>Project Number:</b>	2009KY134B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Kentucky 6th
<b>Research Category:</b>	Not Applicable
<b>Focus Category:</b>	None, None, None
<b>Descriptors:</b>	technology transfer, symposium, internet
<b>Principal Investigators:</b>	Lindell Ormsbee, Anna Goodman Hoover, Jim Kipp

### Publication

1. Proceedings Kentucky Water Resources Annual Symposium, March 22, 2010, Kentucky Water Resources Research Institute, Lexington, Kentucky, 94 p.

## **Kentucky Information Transfer Activities (2009KY134B)**

### **Problem and Objectives**

The Water Resources Research Act requires that Institutes or Centers shall:

- (1) plan, conduct, or otherwise arrange for competent applied and peer reviewed research that fosters –
  - (A) improvements in water supply reliability;
  - (B) the exploration of new ideas that –
    - (i) address water problems; or
    - (ii) expand understanding of water and water-related phenomena;
  - (C) the entry of new research scientists, engineers, and technicians into water resources fields; and
  - (D) the dissemination of research results to water managers and the public.
- (2) cooperate closely with other colleges and universities in the State that have demonstrated capabilities for research, information dissemination, and graduate training in order to develop a statewide program designed to resolve State and regional water and related land problems.

Each institute shall also cooperate closely with other institutes and other organizations in the region to increase the effectiveness of the institutes and for the purpose of promoting regional coordination.

Kentucky information transfer activities are conducted in support of these objectives.

### **Methodology**

Information transfer activities are an important part of the overall program of the Kentucky Water Resources Research Institute. There are two main components, an annual symposium and the institute web site (including the electronic newsletter). The Institute also participates in and supports other technology and information transfer activities throughout the year.

The Associate Director develops the program for the Annual Water Resources Symposium. Presentations in both platform and poster format allow for researchers and practitioners to share progress on planned, ongoing, and completed water-related activities through the Commonwealth each year.

The Information Specialist Senior assists with creating program announcements and the proceedings volume for the symposium. She also prepares information for the electronic newsletter. She develops and maintains content for several web sites including the main Institute page at: [www.uky.edu/WaterResources/](http://www.uky.edu/WaterResources/). Links for additional sites describing projects and activities (for example volunteer sampling results and watershed

pages for the Kentucky River basin) are provided on the main web site. Research translation to make results accessible for a variety of audiences is a major goal for all of the technology transfer activities of the unit.

The Institute cooperates closely with other groups and agencies in planning additional technology transfer activities in the Commonwealth. These efforts included support for seminar/lectures, other web sites, an open house during Earth Science Week, and a weeklong summer camp for high school sophomores from eastern Kentucky counties. Institute staff members serve a variety of support roles on technical committees and advisory panels for agencies and volunteer organizations to help disseminate relevant information about ongoing activities and research results;

### **Principal Accomplishments and Activities**

Kentucky Water Awareness Month is an educational program of the University of Kentucky Cooperative Extension Service, Environmental and Natural Resources Issues Task Force (the Associate Director of KWRRI is a member). The program promotes overall water awareness for citizens of Kentucky during May each year. Materials are developed by a committee at the state level and distributed to all of the 120 county extension offices in the state. Individual county agents are encouraged to tailor the program to fit their county's specific needs and to use the materials to enhance their program efforts. The materials remain available throughout the year for use by classroom teachers, 4-H volunteers, and others interested in water issues through the ENRI internet site: [www.ca.uky.edu/enri/](http://www.ca.uky.edu/enri/)

The Robinson Scholars Program serves first generation college-bound students from 29 eastern Kentucky counties who have demonstrated the potential to succeed, but who might encounter social, economic, cultural, or institutional impediments to completion of a four-year college degree. The program provides general support, leadership development opportunities, and a University of Kentucky scholarship upon graduation from high school. The Water Pioneers Water Quality Initiative was developed by KWRRI for rising high school sophomores in the program. It is held for 5 days in June and immerses the teens in activities designed to open their eyes to the importance of healthy watersheds using a diverse curriculum designed to show nature's interconnectivity. Following the camp, the students use knowledge that they gain to partner with educators, volunteers, and other interested groups in their home counties to increase awareness of best management practices for water quality through a community service/outreach project of their own design.

The Kentucky Water Resources Research Institute and the University of Kentucky Department of Earth and Environmental Sciences co-sponsored the Geological Society of America Hydrology Division Birdsall-Dreiss Distinguished Lecture "Will China Run Out of Water" by Dr. Chunmiao Zheng on April 16, 2009.

The Kentucky Water Resources Research Institute and the University of Kentucky Department of Earth and Environmental Sciences co-sponsored the National Ground Water Association Henry Darcy Distinguished Lecture “Environmental Tracers in Modern Hydrology: Reducing Uncertainty in Ground Water Flow Estimation” by Dr. Peter Cook on April 30, 2009.

An open house was held on Wednesday evening 10/07/2009 during Earth Science Week. This event was co-sponsored with the Kentucky Geological Survey. KWRRI staffed a water exhibit for the elementary, middle school, and high school students and their parents who attended the event (approximately 200 people).

KWRRI continues participation in the Bluegrass Partnership for a Green Community cooperative effort between the University of Kentucky, the Fayette County public school system, and the Lexington-Fayette Urban County Government, and numerous other partners including a number of citizen watershed groups in the community. Staff members are active with the water/stormwater team.

Cyberseminars provided through the Consortium for the Advancement of Hydrologic Sciences, Inc. were made available by KWRRI on the University of Kentucky campus for interested faculty, staff, students, and local professionals.

The Kentucky Water Resources Annual Symposium was held on March 22, 2010. Although the date of the symposium fell outside of FY2009, most of the planning and preparation occurred during the fiscal year. Two concurrent sessions provided time slots for 26 oral platform presentations. Twenty-one posters were also presented during a separate poster session. The 6 student research enhancement projects funded during FY2009 presented their results. Approximately 115 people attended the meeting. Abstracts for all of the presentations were distributed to participants on the day of the meeting: Proceedings of the Kentucky Water Resources Annual Symposium, 2010, Kentucky Water Resources Research Institute, Lexington, Kentucky, 94 p. The full proceedings document is also available free of charge online through a link on the institute web site. The document includes contact information for all authors and presenters and an abstract for each presentation. Symposium participants also receive a list of attendees providing basic contact information for each individual who pre-registered for the symposium. Attendees include researchers, personnel from local, state, and federal agencies, undergraduate and graduate students, participants from volunteer groups and NGOs, and members of the general public. Conference registration fees are kept low through partial subsidy of symposium expenses (using 104(b) technology transfer and matching funds) to ensure accessibility to individuals from all potential audiences.

Maintenance of the institute web site provides open access for those interested in the activities of the Institute as well as providing links to related sites and information maintained by others. Creation and maintenance of the web site are ongoing throughout the year. Links on the site provide direct access to the Association of State Dam Safety

Officials, the Kentucky Research Consortium for Energy and the Environment, the Kentucky River Watershed Watch Sampling Database, the National Institutes for Water Resources, PRIDE, the UK Superfund Basic Research Program Research Translation Core and the Kentucky River Watershed page. The Institute's newsletter WATERWORKS is also available in electronic format through a link on the web page.

# **USGS Summer Intern Program**

None.

<b>Student Support</b>					
<b>Category</b>	<b>Section 104 Base Grant</b>	<b>Section 104 NCGP Award</b>	<b>NIWR-USGS Internship</b>	<b>Supplemental Awards</b>	<b>Total</b>
<b>Undergraduate</b>	3	0	0	0	3
<b>Masters</b>	3	0	0	0	3
<b>Ph.D.</b>	3	0	0	0	3
<b>Post-Doc.</b>	0	0	0	0	0
<b>Total</b>	9	0	0	0	9

## Notable Awards and Achievements

2009KY126B - The experience gained through this student research enhancement grant was a key factor in the student's competitiveness for an internship with the U.S. EPA Office of Pesticide Programs, which she began in September 2009. In this capacity, the student was appointed as a branch representative for ongoing deliberations regarding the U.S. EPA Endocrine Disruptor Screening Program and was nominated by the Senior Scientist to act as a divisional liaison for matters pertaining to the development of evaluation protocols for endocrine data submissions. The graduate student also received a \$2,500 graduate research award from the Watersheds Studies Institute at Murray State University. In support of her primary research, the student coordinated infrastructure, developed and validated assays, and refined protocols for amphibian hormone analysis that will benefit future research at Murray State University. The lab now has capacity to analyze approximately 1,000 additional samples.

2009KY130B - Entomological Society of America President's Prize, Second Place for Oral Presentations for: Adkins, J.K. and L.K. Rieske-Kinney. The role of eastern hemlock on headwater stream processes: Benthic and riparian consequences of hemlock woolly adelgid invasion. Entomological Society of America National Meeting. December 2009



## **Publications from Prior Years**

1. 2003KY17B ("Biochemical and hormonal effects of incomplete site remediation: evaluating resident fish species") - Articles in Refereed Scientific Journals - Brammell, B.F., J.S. McClain, J.T. Oris, D.J. Price, W.J. Birge, and A.A. Elskus, 2009, CYP1A Expression in Caged Rainbow Trout Discriminates Among Sites with Various Degrees of Polychlorinated Biphenyl Contamination, Archives of Environmental Contaminant Toxicology, Springer Science+Business Media, LLC, Published online: 07 August 2009